REVIEW

Clinical outcome of patients with *Helicobacter pylori* infection: the bug, the host, or the environment?

S N Sgouros, C Bergele

Postgrad Med J 2006;**82**:338–342. doi: 10.1136/pgmj.2005.038273

It is well established that only a minority of patients with *Helicobacter pylori* infection develop severe inflammation leading to peptic ulcer or gastric cancer. Recent evidence suggests that the virulence factors of the organism do not seem crucial in the progression of inflammation towards a more severe disease. It seems probable that other host derived and environmental factors are more significant in determining clinical outcome but additional studies are needed to clarify the underlying mechanisms involved in the pathogenesis of infection.

Helicobacter pylori is a micro-aerophilic, Gram negative, slow growing, spiral shaped, and flagellated organism. Its most characteristic enzyme is a potent multi-subunit urease that is crucial for its survival at acidic pH and for its successful colonisation of the gastric environment, an area that few other microbes can colonise. *H pylori* infection is probably the most common chronic bacterial infection of humans, present in almost half of the world population.[1] The presence of the bacterium in the gastric mucosa is associated with chronic active gastritis and is implicated in more severe gastric diseases, including chronic atrophic gastritis (a precursor of gastric carcinomas), peptic ulceration, and mucosa associated lymphoid tissue (MALT) lymphomas.

Because of its importance as a human pathogen investigators have sequenced the complete genome of two representatives *H pylori* strains (26695 and J99) by the whole genome random sequencing method.[2 3] Comparing *H pylori* genes with genes of known function in other bacteria gave immediate insights into *H pylori* metabolism, structure, adaptive mechanisms, and virulence. In addition, comparison of the genomic sequence of the two independent clinical isolates has shown that they are highly conserved, with only 7% of the proteins being strain specific.

The pathogenesis of *H pylori* associated gastroduodenal disease remains poorly understood. It is clear that only a minority of infected people develop severe inflammation leading to peptic ulcer or gastric cancer. What are the factors that decide if an infected person will develop severe disease?

See end of article for authors' affiliations

Correspondence to: Dr S N Sgouros, Nafpaktias 5, Agia Paraskevi, 15341, Athens, Greece; spisgon@otenet.gr

Submitted 10 June 2005
Accepted 22 July 2005

H PYLORI RELATED FACTORS

Virulence factors of *H pylori* may be divided into colonisation factors, factors that allow it to evade host defence, and factors that are responsible for tissue injury.

Colonisation factors

Colonisation factors are attributes of an organism that allow it to establish its presence and to persist despite the host's attempts to rid himself of infection.

Flagella and motility

H pylori has been shown to require flagella for infection of the stomach. Flagella allow the bacterium to swim across the viscous gastric mucus and reach the more neutral pH below the mucus. To analyse whether flagella themselves or motility is needed by these pathogens, investigators constructed flagellated non-motile mutants. Their results support a model in which motility is used for the initial colonisation of the stomach and also to attain full infection levels.[4]

Urease system

H pylori synthesises urease constitutively. As urease hydrolyses urea to form ammonia and carbon dioxide, and ammonia can absorb acid to form ammonium, it is natural to suspect that this dedication to make urease has a relation to survival and growth in the acidic environment of human stomach. This suspicion has been confirmed in animal models but it is not certain that the requirement for urease is for colonisation as well as for infection.[5]

There are data showing that the organisms do buffer their periplasm that lies between their inner and outer membrane, in acidic pH, using their intrabacterial urease activity.[6] In contrast with surface or free urease, measurement of intrabacterial urease activity at different pH values, shows low urease activity at neutral pH, rapid increase between pH 6.0 and 5.0, and steady activity down to a pH of 2.5 but still present at pH 2.0.[7] Expression of the *H pylori* ureI gene is required for acidic pH activation of cytoplasmic urease.[8]

Adhesins

H pylori selectively binds to gastric epithelial cells and its colonisation of the digestive tract is limited to areas lined by gastric type epithelial cells. On adhesion, tyrosine phosphorylation and cytoskeletal rearrangement occurs, leading to a remodelling of the apical surface of the epithelial cells.[9] Several epithelial structures have been

Abbreviations: MALT, mucosa associated lymphoid tissue; IL, interleukin; Hsp, heat shock protein; LPS, lipopolysaccharides; Hp-NAP, neutrophil activating protein of *H pylori*; cagA, cytotoxin associated gene A

implicated in adhesion, including lipids, gangliosides, and sulphated carbohydrates, but to date the adhesins on the bacterial surface that bind to the epithelium are poorly understood.

As of yet, no adhesions have been confirmed as being important for in vivo survival of *H pylori*. With the sequence of the *H pylori* genome in hand, it should be possible to more easily determine the role of specific genes in virulence. Genes of immediate interest are the OMPs, which may undergo phase and antigenic variation and may represent adhesions.[10]

Adhesion is necessary for the initiation of the inflammatory cascade. In particular adhesion is a prerequisite for interleukin 8 (IL8) secretion by gastric epithelial cells.[11] Adherence also promotes the development of more severe disease. BabA adhesin binds the Lewis b blood group antigen on the gastric epithelium and is associated with duodenal ulcer, distal gastric cancer and more severe gastritis.[12]

Other factors that may also participate in *H pylori* adhesion are AlpA and AlpB. Both of these are required for adhesion to human gastric tissue sections.[13] BabA and Alp proteins are members of the large family of related outer membrane proteins (Hop proteins). These proteins are not present in all strains of *H pylori* and thus may represent means by which the pathogen gains control of the host response.

A recent described adhesin is a sialic acid binding adhesin (SabA). The ability of many *H pylori* strains to adhere to sialylated glycoconjugates expressed during chronic inflammation might contribute to virulence and the extraordinary chronicity of *H pylori* infection.[14]

Heat shock proteins (Hsp)

H pylori expresses two Hsp, A and B. They are highly antigenic. The clinical outcomes of *Hpylori* infection are not related to HspA antigenicity or to sequence variation.[15] Recent data suggest that a common epitope is present in human hsp60 and its bacterial homologue hspB.[16] Thus infection with *H pylori* may induce antibodies against bacterial hspB that cross react with human hsp60, through the molecular mimicry of these proteins. On the other hand, it is well established that the immune response to hsp60 is closely associated with MALT lymphoma.[17] Currently patients with gastric disease other than MALT lymphoma and increased IgG titres to hsp60 are under careful follow up to see whether they will develop gastric MALT lymphoma.[16] If this occurs it seems reasonable to hypothesise that hspB is closely associated to pathogenesis of MALT lymphoma.

Metal acquisition proteins

Adaptation of *H pylori* to the conditions in the gastric mucosa includes acquisition mechanisms that overcome a temporary lack of the metals iron, nickel, and zinc. Iron is essential for maintaining the basic energy and redox metabolism, whereas nickel is an essential cofactor of urease, an important virulence determinant of *H pylori*. However, as overacquisition of iron, nickel, and other metals is deleterious, the control mechanisms regulating the intracellular availability of these metals are of crucial importance.

Iron responsive regulation in prokaryotes is usually mediated through the ferric uptake regulator (Fur) protein. Fur homologs downregulate the expression of genes involved in iron uptake when the cytoplasmatic ferrous iron concentration increases, thus abolishing iron acquisition. Iron responsive regulation has been seen in *H pylori*, and genetic analysis showed that *H pylori* possesses a Fur homologue.[18] The *H pylori* ferritin protein Pfr is a member of the non-heme ferritin subfamily, all of which store iron in the inner space of a multimeric protein shell consisting of 24 identical subunits. The protein plays a substantial part in the storage of iron and protects the bacteria from metal toxicity.[19] Ferritins thus catalyse a function that is the exact opposite of that of iron uptake systems, which increase the cytoplasmic iron concentration.

Induction of hypochlorhydria

It is well established that acute infection is accompanied by transient hypochlorhydria.[20] Suggestions for the mechanism by which *H pylori* increases the gastric pH include: (1) presence of acid neutralising substances (ammonia) in the infected gastric mucosa, (2) increased levels of cytokines such as IL1b, which is known to inhibit gastric acid secretion, (3) exposure of parietal cells to acid inhibitory substances released by *H pylori*.

H pylori interferes with parietal cell acid production by two mechanisms: (1) the bacterium increases proton permeability at the secretory membrane of the parietal cell (it causes back diffusion of protons from the secretory canaliculus into the cytosol of the parietal cell), and (2) in addition inhibits H^+/K^+-ATPase activity.[21]

Factors that allow organism to evade host defence

The bacterium possesses a well defined battery of virulence factors that allow it to evade host defence. These are shedding of surface proteins, catalase, superoxide dismutase, and poorly reactive lipopolysaccharide.

It has been shown that after successful colonisation some bacteria are killed by host defensive mechanisms resulting in shedding of their surface proteins. These proteins are connected to receptors on the surface of other bacteria and bind cytokines and immunoglobulins. This has been interpreted as an indirect defensive mechanism of *H pylori* to evade host defence.

Despite the fact that the organism is an obligate aerobe, it is unable to grow in atmospheric concentrations of oxygen. Microaerophilic organisms, like *H pylori*, are particularly vulnerable to the detrimental effects of oxygen and oxidative stress. Nevertheless, they do possess some of the enzymatic machinery needed to eliminate or minimise toxic oxygen derived products. These enzymes are superoxide dismutase, catalase, and several putative peroxidases.[22]

It is well known that bacterial lipopolysaccharides (LPS) may induce both strong local and systemic inflammation in animals as well as humans, and therefore, *H pylori* LPS is one of the factors that could potentially influence local gastric inflammation and the clinical outcome during an *H pylori* infection. In general, *H pylori* LPS is much less potent in activation of inflammatory cells than LPS from members of the family enterobacteriaceae, for example, *Escherichia coli* and *Salmonella spp*. Despite its comparatively low toxic activity, *H pylori* LPS has been shown to activate inflammatory cells to produce different cytokines and chemokines, such as TNFα, IL8, IL1, and monocyte chemotactic protein-1.[23] In addition, the LPS of some strains contains structures identical to the fucosylated Lewis x and Lewis y blood group antigens expressed on the gastric mucosa. The antigenic mimicry may result in immune tolerance against antigens of the pathogen or in induction of autoantibodies that recognise gasric epithelial cells, frequently seen in patients with chronic active gastritis.[24]

Key points 1

H pylori colonisation factors do not seem crucial in the pathogenesis of infection. Adhesins (mainly BabA) are associated with more severe inflammation but this requires further research.

Key points 2

Bacterial lipopolysaccharides might induce autoantibodies that are implicated in the pathogenesis of chronic active gastritis.

Factors that induce tissue injury

The vacuolating cytotoxin A (vacA)

The vacuolating toxin (VacA) is an important determinant of *H pylori* associated gastric disease. The association of vacA with peptic ulcer disease, MALT lymphoma, and gastric cancer has been well validated, at least in Europe where the background population has a low incidence of type I strains (defined as cagA and vacA positive).[25]*H pylori* vacA s1 strains have been associated with the occurrence of peptic ulcer disease[26] and vacA m2 allele is also associated with peptic ulcer disease and gastric cancer.[27] The original hypothesis was that the s1 genotype was associated with duodenal ulcer disease and the s2 genotype had low ulcerogenic potential. Data are now overwhelming that vacA genotyping is not useful to predict symptoms, presentation, response to therapy, or degree of inflammation. VacA genotyping is useful to predict cagA status.[28]

The mechanism of action of vacA has recently been further described. Binding of free or membrane bound vacA to epithelial cells is receptor mediated. VacA forms pores in lysosomal membranes, increasing anion permeability and generating vacuoles.[29] In addition, vacA has been shown to reduce transepithelial resistance by loosening tight junctions.[30] Finally, vacA inhibits de novo antigen binding by MHC class II receptor, a mechanism that can contribute to a down regulation of the host immune response, which has been correlated in mice with increased gastritis and atrophy.[31]

The neutrophil activating protein of *H pylori* (*H pylor*-NAP)[32]

H pylori-NAP has been shown to be chemotactic for neutrophils and monocytes. It induces the production of oxygen radicals in human neutrophils via a cascade of intracellular activation events that may contribute to the damage of the stomach mucosa. This protein has recently been shown to be an important antigen in the human immune response to *H pylori* infection, making it a strong vaccine candidate. In addition, mice vaccinated with recombinant *H pylori*-NAP were protected against *H pylori* challenge. A number of other reports have proposed that *H pylori*-NAP acts as an adhesin being capable of binding several different compounds in vitro.

The cytotoxin associated gene A (cagA) and the cag associated pathogenicity island (cag-PAI)

CagA is the product of one gene from cag-PAI and is involved in the cytoskeletal changes and host proteins dephosphorylation that occur when a cagA positive strain adheres to host cell. The cag-PAI is a type IV secretory apparatus that injects cagA into the host cell and is involved in the induction of cytokine expression in gastric epithelial cells, which is seen as a pronounced increase in IL8 expression.[33] Cytokine induction associated with the cag-PAI is independent of cagA. The signal transduction pathways are thought to be through nuclear factor kB (NF-kB) and activator protein 1 (AP1). Before activation, NF-Kb, resides in the cytoplasm and upon activation it translocates to the nucleus, where it binds to DNA at kB sites and upregulates IL8 gene production.[34]

People infected with *H pylori* who have a functional cag-PAI have increased mucosal concentrations of IL8, pronounced neutrophilic infiltration into the gastric mucosa, and a theoretically increased risk of developing peptic ulcer and gastric cancer. However, in East Asia where more than 90% of isolates possess the cag-PAI, a relation of the cag-PAI and clinical outcome has not been reported. Conversely, in Western countries, where *H pylori* strains lacking cag-PAI are found in higher percentage, there are data showing increased likelihood of symptomatic outcome.[35] Nevertheless, the presence of a functional cag-PAI has no predictive value regarding current or future clinical presentation. *H pylori* strains lacking a functional cag-PAI are not commensal as they are also found in patients with peptic ulcer disease or gastric cancer, only at lower frequency.

IceA

IceA is a gene that is induced by contact with epithelium. The gene product is unknown but it seems to be a bacterial restriction enzyme. There are two variants of the iceA gene, iceA1 and iceA2. The initial studies suggested that iceA1 was correlated with duodenal ulcer.[36] More recent studies are conflicting. In a large study involving four different countries (USA, Colombia, Japan, and Korea), to avoid the regional variation of *H pylori* genes, the results failed to confirm an association between iceA1 and clinical outcome,[37] but a more recent large study in Japan showed that the iceA1 allele is associated with increased gastric inflammation.[38]

HOST RELATED FACTORS

Several laboratories have provided evidence that the host response is an important determinant in *H pylori* associated disease progress. An alternative model of *H pylori* associated disease is the *H felis* mouse model, which has been extensively used to examine how the host response prevents and/or exacerbates *H pylori* induced gastroduodenal disease. In the mouse *H felis* infection model, several inbred strains of mice, exhibit severe inflammation/gastric atrophy (''high responders''), in contrast with others that are low gastritis/atrophy responders to *H felis* infection.[38] These results suggest that the nature of the host immune or inflammatory response to *H pylori* infection in humans might be more important in determining disease outcome than *H pylori* virulence factors.

In concordance to this hypothesis is the fact of rapid change worldwide in the incidence of gastric cancer and duodenal ulcer disease. This might be explained from a similar decrease in the prevalence of a particular virulence factor. However, several studies evaluating the prevalence of putative virulence factors in different birth cohorts have shown that this is not the case.[39]

Genetic susceptibility to infection has been reported from large epidemiological studies, which implies that the host response may be regulated from genetically determined factors. There are data from developed countries such as USA, which exhibit different prevalence among different ethnic groups of similar socioeconomic status.[40] Similar findings come from South East Asian countries in which the Malays have been shown to have consistently low prevalence compared with the Indians and Chinese.[41] These data show a racial linked genetic susceptibility to infection. Genetic susceptibility has been confirmed also, in studies showing that monozygotic twins reared apart or together had a higher rate of concordance of infection than did age matched dizygotic twins.[42]

Key points 3

Despite the results of initial studies from developed countries, *H pylori* factors that induce tissue injury do not correlate with a more severe clinical outcome.

One small study has shown significant association between the prevalence of the HLA-DQ5 genotype and *H pylori* infection with accompanying atrophic gastritis or intestinal metaplasia while investigations of the HLA-DQA1*0102 genotype noted a lower prevalence of DQA1*0102 among patients with gastric cancer and coexisting *H pylori* infection. This work in HLA may be pointing in an interesting direction but requires much larger studies, adjusted appropriately for the multiple comparisons being made, before any conclusions can be drawn.[43]

A host related factor that has been shown to predict disease progression is the size of parietal mass at the time of exposure to *H pylori*.[44] Those with a large cell mass and high acid output have an infection confined to the antrum, where the environment is less acidic and favours *H pylori* colonisation. These patients have antrum predominant gastritis and are likely to develop duodenal ulcers. To date this unique response to *H pylori* infection has not been linked to a distinct cytokine response; duodenal ulcer disease seems to require both hypersecretion of gastric acid and the activity of proinflammatory cytokines.[45]

On the other hand, for those with a small cell mass, acid production is insufficient to protect the corpus from infection and subsequent cellular degeneration compromises acid output still further. This favours the loss of specialised glandular cell types such as parietal and chief cells and the development of corpus predominant atrophy, which seems to be a critical initiating step in the progression towards gastric cancer.[45]

Other host related factors that have been shown to predict disease progression towards gastric cancer, are increased gastrin levels at the time of exposure to *H pylori*,[46] and single nucleotide polymorphisms in the gene encoding IL1b.[47] It is probable that single nucleotide polymorphisms in other genes encoding cytokines or cytokine receptors that influence the risk of gastric atrophy and cancer will be found.

ENVIRONMENTAL FACTORS

It is well established that environmental factors may also affect clinical outcome of *H pylori* infection. For example, migrating from a region with high prevalence of gastric cancer to a region with low prevalence did not reduce the rate of cancer in the migrants but resulted in an important reduction in risk for their offspring, suggesting that the environment is more important than genetics in determining the clinical outcome of an *H pylori* infection.

The environmental factors that seem most important in determining the pattern of gastritis (and thus the risk of any of the different *H pylori* outcomes) are the presence of childhood febrile illnesses and diet.

Childhood infections such as tonsillitis, infectious diarrhoeas, and diphtheria are associated with a pronounced decrease in acid secretion. Low acid secretion in childhood occurs also in malnutrition. Thus, regions where childhood infections and malnutrition are common would provide the ideal environment for *H pylori* colonisation and the development of corpus predominant atrophy, as discussed above. Diphtheria is especially prone to cause gastric damage and may even be a cause of gastric atrophy. Indeed, there are speculations considering that immunisation against diphtheria played an important part in the prevention of early onset atrophic gastritis and therefore, of gastric cancer.[48]

Key points 4

Genetic susceptibility to infection and single nucleotide polymorphisms in genes encoding cytokines or cytokine receptors might influence the clinical outcome of *H pylori* infection.

Key points 5

Epidemiological data suggest that dietary habits might influence the severity and clinical outcome of *H pylori* infection.

However, in regions where childhood infectious diseases, malnutrition and *H pylori* infection are all common, one would expect a high frequency of an accelerated development of corpus gastritis. This is not a universal finding suggesting that a number of other factors may also be important in determining whether atrophic gastritis develops after *H pylori* infection. In these regions there is a year round availability of fresh fruits and vegetables. Investigators speculated that ingestion of fresh fruits and vegetables might retard the development of gastric atrophy (the ''banana hypothesis'').

There is some evidence that establishes a long suspected correlation between salt intake, *H pylori*, and gastric cancer risk. In the Intersalt study,[49] authors note that, where measured appropriately, salt intake levels in African countries are considerably lower than in most other countries and they suggest that salt might be the permissive cofactor that is required for *H pylori* infection to act as a cancer risk factor.

Recent data suggest that some dietary habits might have antihelicobacter activity such as mastic gum (1 mg per day for two weeks)[50] or Chinese tea.[51]

CONCLUSIONS

H pylori is a common bacterial pathogen that colonises the gastric mucosa of over 50% of the world's population. All infected people exhibit chronic gastric inflammation, and about 1% of patients develop gastric cancers, including adenocarcinomas and MALT lymphomas. In 1994, the World Health Organisation International Agency for Research on Cancer classified *H pylori* as a type I, or definite carcinogen. Because the prevalence of gastric inflammation among *H pylori* infected patients varies between persons, countries, and geographical areas, *H pylori* disease related outcomes are believed to be determined by an interplay between bacterial factors, host factors, and their interaction with the environment.

Through novel techniques and experimental approaches, a great deal of progress has been made in our understanding of *H pylori* induced gastric inflammation. Although infection with *H pylori* is known to be a prerequisite for promoting peptic ulcer disease and gastric cancers, it has become increasingly clear that, in addition to the bacteria, host and environmental factors are involved. Elucidating these factors and delineating how they work together may ultimately lead to the development of novel therapeutic targets to combat these diseases.

Initially, there were data showing a clear predominance of *H pylori* virulence factors on human's disease outcome but additional studies, mainly from East Asia, failed to support this model. It seems probable that other host derived and environmental factors are more significant in determining clinical outcome, but additional studies are needed to evaluate the underlying pathophysiological mechanisms involved in the clinical outcome of infection.

Authors' affiliations
S N Sgouros, Department of Gastroenterology, Athens Naval and Veterans Hospital, Athens, Greece

C Bergele, 2nd Department of Gastroenterology, ''Evangelismos'' General Hospital, Athens, Greece

Funding: none.

Competing interests: none declared.

REFERENCES

1 **Cover TL**, Blaser MJ. Helicobacter pylori infection, a paradigm for chronic mucosal inflammation: pathogenesis and implications for eradication and prevention. *Adv Int Med* 1996;**41**:85–117.
2 **Tomb JF**, White O, Kerlavage AR, *et al*. The complete genome sequence of the gastric pathogen Helicobacter pylori. *Nature* 1997;**388**:539–47.
3 **Alm RA**, Ling LS, Moir DT, *et al*. Genomic sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori. *Nature* 1999;**397**:176–80.
4 **Ottemann MK**, Lowenthal AK. Helicobacter pylori uses motility for initial colonization and to attain robust infection. *Infect Immun* 2002;**70**:1984–90.
5 **Andrutis KA**, Fox JG, Schauer DB, *et al*. Inability of an isogenic urease negative mutant strain of Helicobacter mustelae to colonize the ferret stomach. *Infect Immun* 1995;**63**:3722–5.
6 **Athmann C**, Zeng N, Kang T, *et al*. Local pH elevation mediated by the intrabacterial urease of Helicobacter pylori cocultured with gastric cells. *J Clin Invest* 2000;**106**:339–47.
7 **Rektorschek M**, Weeks D, Sachs G, *et al*. Influence of pH on metabolism and urease activity of Helicobacter pylori. *Gastroenterology* 1998;**115**:628–41.
8 **Scott DR**, Marcus EA, Weeks DL, *et al*. Expression of the *Hp urel* gene is required for acidic pH activation of cytoplasmic urease. *Infect Immun* 2000;**68**:470–7.
9 **Segal ED**, Falkow S, Tomkins LS. Helicobacter pylori attachment to gastric cells induces cytoskeletal rearrangements and tyrosine phosphorylation of host cell proteins. *Proc Natl Acad Sci U S A* 1996;**93**:1259–64.
10 **Mc Gee DJ**, Mobley HL. Mechanisms of Helicobacter pylori infection; bacterial factors. *Curr Top Microbiol Immunol* 1999;**241**:155–80.
11 **Keates S**, Hitti YS, Upton M, *et al*. Helicobacter pylori infection activates NF-kappaB in gastric epithelial cells. *Gastroenetrology* 1997;**113**:1099–109.
12 **Gerhard M**, Lehn N, Neumayer N, *et al*. clinical relevance of the Helicobacter pylori gene for blood group antigen-binding adhesin. *Proc Natl Acad Sci U S A* 1999;**96**:12778–83.
13 **Odenbreit S**, Till M, Hofreuter D, *et al*. Genetic and functional characterization of the alpAB gene locus essential for the adhesion of Helicobacter pylori to human gastric tissue. *Mol Microbiol* 1999;**31**:1537–48.
14 **Mandavi J**, Sonden B, Hurtig, *et al*. Helicobacter pylori SabA adhesin in persistent infection and chronic inflammation. *Science* 2002;**297**:573–8.
15 **Ng EKW**, Thompson SA, Pérez-Pérez GI, *et al*. Helicobacter pylori heat shock protein A: serologic responses and genetic diversity. *Clin Diagn Lab Immun* 1999;**6**:377–82.
16 **Kawahara Y**, Yokota K, Mizuno M, *et al*. Antibodies to human gastric epithelial cells and heat shock protein 60 in Helicobacter pylori positive mucosa associated lymphoid tissue lymphoma. *Gut* 1999;**45**:20–3.
17 **Yamaguchi H**, Osaki T, Kai M, *et al*. Immunoglobulin G1 antibody response to Helicobacter pylori heat shock protein 60 is closely associated with low-grade gastric mucosa-associated lymphoid tissue lymphoma. *Clin Diagn Lab Immun* 2001;**8**:1056–9.
18 **Escolar L**, Perez-Martin J, de Lorenzo V. Opening the iron-box: transcriptional metalloregulation by the Fur protein. *J Bacteriol* 1999;**181**:6223–9.
19 **Waidner B**, Greiner S, Odenbreit S, *et al*. Essential role of ferritin Pfr in Helicobacter pylori iron metabolism and gastric colonization. *Infect Immun* 2002;**70**:3923–9.
20 **Harford WV**, Barnett C, Lee E, *et al*. Acute gastritis with hypochlorhydria: report of 35 cases with long term follow up. *Gut* 2000;**47**:467–72.
21 **Beil W**, Sewing KF, Busche R, *et al*. Helicobacter pylori augments the acid inhibitory effect of omeprazole on parietal cells and gastric H^+/K^+-ATPase. *Gut* 2001;**48**:157–62.
22 **Olczak AA**, Olson JW, Maier RJ. Oxidative-stress resistance mutants of Helicobacter pylori. *J Bacteriol* 2002;**184**:3186–93.
23 **Yamaoka Y**, Kita M, Kodama T, *et al*. Chemokines in the gastric mucosa in Helicobacter pylori infection. *Gut* 1998;**42**:609–17.
24 **Covacci A**, Telford JL, DelGiudice G, *et al*. Helicobacter pylori virulence and genetic geography. *Science* 1999;**284**:1328–33.
25 **de Figueiredo Soares T**, de Magalhaes Queiroz DM, Mendes EN, *et al*. The interrelationship between Helicobacter pylori vacuolating cytotoxin and gastric carcinoma. *Am J Gastroenterol* 1998;**93**:1841–7.
26 **Miehlke S**, Meining A, Morgner A, *et al*. Frequency of vacA genotypes and cytotoxin activity in Helicobacter pylori associated with low-grade gastric mucosa associated lymphoid tissue lymphoma. *J Clin Microbiol* 1998;**36**:2369–70.
27 **Pagliaccia C**, de Bernard M, Lupetti P, *et al*. The m2 form of the Helicobacter pylori cytotoxin has cell type specific vacuolating activity. *Proc Natl Acad Sci U S A* 1998;**95**:10212–17.
28 **Yamaoka Y**, Kodama T, Kita M, *et al*. Relationship of vacA genotypes of Helicobacter pylori to cagA status, cytotoxin production, and clinical outcome. *Helicobacter* 1998;**4**:241–53.
29 **de Bernard M**, Burroni D, Papini E, *et al*. Identification of the Helicobacter pylori vacA toxin domain active in the cell cytosol. *Infect Immun* 1998;**66**:6014–16.
30 **Papini E**, Satin B, Norais N, *et al*. Selective increase of the permeability of polarized epithelial cell monolayers by Helicobacter pylori vacuolating toxin. *J Clin Invest* 1998;**102**:813–20.
31 **Sutton P**, Wilson J, Genta R, *et al*. A genetic basis for atrophy: dominant non-responsiveness and Helicobacter induced gastritis in F1 hybrid mice. *Gut* 1999;**45**:335–40.
32 **Dundon WG**, Nishioka H, Polenghi A, *et al*. The neutrophil-activating protein of Helicobacter pylori. *Int J Med Microbiol* 2002;**291**:545–50.
33 **Censini S**, Lange C, Xiang Z, *et al*. CagA pathogenicity island of Helicobacter pylori encodes type-I specific and disease associated virulence factors. *Proc Natl Acad Sci U S A* 1996;**93**:14648–53.
34 **Naumann M**, Wessler S, Bartsch C, *et al*. Activation of activator protein 1 and stress response kinases in epithelial cells colonized by Helicobacter pylori encoding the cag pathogenicity island. *J Biol Chem* 1999;**274**:31655–62.
35 **Yamaoka Y**, Kodama T, Gutierrez O, *et al*. Relationship between Helicobacter pylori iceA, cagA, and vacA status and clinical outcome: studies in four different countries. *J Clin Microbiol* 1999;**37**:2274–9.
36 **van Doorn LJ**, Figueiredo C, Sanna R, *et al*. Clinical relevance of cagA, vacA and iceA status of Helicobacter pylori. *Gastroenterology* 1998;**115**:58–66.
37 **Nishiya D**, Shimoyama T, Fukuda S, *et al*. Evaluation of the clinical relevance of the iceA1 gene in patients with Helicobacter pylori infection in Japan. *Scand J Gastroenterol* 2000;**35**:36–9.
38 **Nedrud JG**, Czinn SJ. Host, heredity and helicobacter. *Gut* 1999;**45**:323–4.
39 **Graham**, Yamaoka Y. Disease specific Helicobacter pylori virulence factors; the unfulfilled promise. *Aliment Pharm Ther* 2000;**5**(suppl 1):S3–9.
40 **Malaty HM**, Evans DG, Evans DJ, *et al*. Helicobacter pylori in Hispanics: comparison with blacks and whites of similar age and socioeconomic class. *Gastroenterology* 1992;**103**:813–16.
41 **Kang JY**, Yeoh KJ, Ho KY, *et al*. Racial differences in Helicobacter pylori seroprevalence in Singapore: correlation with differences in peptic ulcer frequency. *J Gastroenterol Hepatol* 1997;**12**:655–9.
42 **Malaty HM**, Engstrand L, Pedersen NL, *et al*. Helicobacter pylori infection: genetic and environmental influences. A study of twins. *Ann Intern Med* 1994;**120**:982–6.
43 **Forman G**. Is there significant variation in the risk of gastric cancer associated with Helicobacter pylori infection? *Aliment Pharm Ther* 1998;**12**(suppl 1):3–7.
44 **McColl KEL**, El-Omar E. Helicobacter pylori and disturbance of gastric function associated with duodenal ulcer disease and gastric cancer. *Scand J Gastroenterol* 1996;**31**(suppl 215):32–7.
45 **Fox JG**, Wang TC. Helicobacter pylori- not a good bug after all. *N Engl J Med* 2001;**345**:829–32.
46 **Wang TC**, Dangler CA, Chen D, *et al*. Synergistic interaction between hypergastrinaemia and Helicobacter infection in a mouse model of gastric cancer. *Gastroenterology* 2000;**118**:36–47.
47 **El-Omar EM**, Carrington M, Chow WII, *et al*. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000;**404**:398–402.
48 **Graham DY**. Helicobacter pylori infection in the pathogenesis of duodenal ulcer and gastric cancer: a model. *Gastroenterology* 1997;**113**:1983–91.
49 **Joosens JV**, Hill MJ, Elliott P, *et al*. Dietary salt, nitrate and stomach cancer mortality in 24 countries. *Int J Epidemiol* 1996;**25**:494–504.
50 **Huwez FU**, Thirlwell D, Cockayne A, *et al*. Mastic gum kills Helicobacter pylori. *N Engl J Med* 1998;**339**:1946.
51 **Yee YK**, Koo MW, Szeto ML. Chinese tea consumption and lower risk of Helicobacter infection. *J Gastroenterol Hepatol* 2002;**17**:552–5.